--31. (new) The method according to claim 27, wherein the method of detection is a quantitative determination method.

--32. (new) The method according to claim 27, wherein the method of detection is a screening method.

--33. (new) A method of obtaining a candidate substance for controlling intimal injury of blood vessels comprising acquiring the candidate substance for controlling intimal injury of blood vessels by screening for a target substance inhibiting granule secretion reaction by the method of claim 32.

--34. (new) The method according to claim 28, wherein the cell line having granule secretion capability is neutrophils or neutrophil-like cultured cells originating from a warm-blooded animal, said neutrophil-like culture cells containing at least one type of granule included in neutrophils.--

B12 Conclude

R E M A R K S

The application has been amended so as to place it in condition for allowance at the time of the next Official Action.

Original claims 1-16 have been canceled and replaced with new claims 17-34. New claims 17-26 correspond respectively with original claims 1-2, 4-6, 8-11, and 13. Claims 27-34 correspond to claims 19-26, the order of steps A and B in claims 19 and 27 being reversed.

The Official Action noted that applicants had not yet provided an English-language translation of the priority document. Attached herewith is that translation whereby applicants perfect their claim to priority under §119.

Applicants acknowledge with appreciation that the previously-withdrawn claims have been rejoined and the requirement for restriction withdrawn.

The Official Action objected to the disclosure due to formal matters. The specification has been carefully reviewed and amended consistent with the formal objections raised. However, see that the term "HEPES" found on specification page 11 is not an abbreviation.

The Official Action rejected claims 1-16 under §112, second paragraph, as being indefinite.

In providing the replacement claims, the originally-filed claims were redrafted so as to remedy the stated bases of rejection. Accordingly, withdrawal of the indefiniteness rejection is respectfully requested.

As to original claim 1, the phrase "an active form of calgranulin" has been replaced with new language.

As to claim 2, the Official Action stated that the recitation of "neutrophil-like culture cells" was indefinite. Claims 2, 6, and 13 (as redrafted) have been amended to specify that the characteristic of these cells is that they contain at least one type of granule included in neutrophils.

In claim 4, the Official Action indicated that the step B recitation of "before, after, or during the step A" rendered the claim indefinite as it is presumed that steps occur in the order listed and accordingly that step B should be practiced during or after and not before step A.

In response to the objection concerning claim 4, claim 4 has been amended to recite that step B can occur after or during step A consistent with the comments of the Official Action. New claim 27 has been introduced which reverses the step A and B order to recite the invention with the step of causing the sample which is suspected to contain the target substance to contact with the cell lines being recited as preceding the step of increasing a calcium binding in the cell lines.

Also concerning claim 4, the Official Action indicated some confusion concerning the recitations of a "substance" and a "subject substance". The claim has been amended to clarify this. See new claim 19, clarifying that there are both a target substance inhibiting or activating a granule secretion reaction and a material secreted from a cell line. See specification page 25, lines 23-27.

The Official Action stated that in claim 5 item B) recites "or" which is said to render the claim indefinite because the order of addition of calgranulin and addition of a water-soluble calcium reagent will result in a different biological effect. Applicants respectfully disagree. Attention is drawn to

the attached material labeled B-1 which shows the order of addition of calgranulin and addition of a water-soluble calcium reagent does not effect the biological activity.

Also see the attached material labeled B-2 which shows that the order of contact between calgranulin, calcium, a target substance (compounds 1-4 of Example 3) and cells does not effect the granule secretion.

Thus, in view of the above, the redrafted claims are believed to address and remedy all of the stated basis of rejection under §112, second paragraph.

The Official Action rejected originally filed claims 1-16 under §112, first paragraph, because the specification was said not to enable a person skilled in the art to make and use the invention commensurate in scope with the claims.

The presently-pending claims are believed to meet all the requirements of §112, first paragraph, and therefore reconsideration and withdrawal of this rejection are respectfully requested.

On page 9 of the Official Action, lines 8-18, there appears to be an "undue experimentation" rejection. From the last sentence of this paragraph, it seems that this rejection is based on the application not providing guidance as to how the modulation of the granule secretion by the target substance actually controls blood vessel injury and related diseases. Accordingly, the claim has been amended so as to recite obtaining

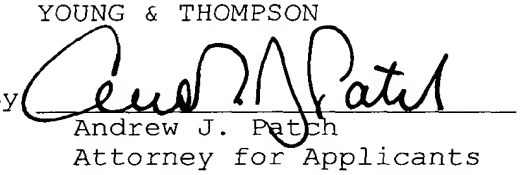
a candidate substance for controlling intimal injury, as opposed to controlling the injury itself. The claim includes the recitation of screening for a target substance inhibiting granule secretion. These recitations are believed to be clearly within the enabled scope of the originally filed application.

In view of the replacement claims addressing both the §112, first and §112 second rejections, the claims are believed to be in condition for allowance.

Attached hereto is a marked-up version of the changes made to the specification. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

YOUNG & THOMPSON

By 

Andrew J. Patch
Attorney for Applicants
Registration No. 32,925
745 South 23rd Street
Arlington, VA 22202
Telephone: 703/521-2297

February 24, 2003

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Page 2, the paragraph beginning on line 22, bridging page 3, has been replaced as follows:

--However, [themechanism] the mechanism of secretion of neutrophil granules is not yet elucidated at present. An increase in the calcium concentration in neutrophils is known to be indispensable for secretion of granules. However, no molecules which are activated by an increase in the calcium concentration and induce granule secretion are known. Therefore, there have been no specific neutrophil secretion inhibitors developed so far, nor any genetic therapy targeting the inhibition of neutrophil secretion inhibitors practiced.--.

Page 9, the paragraph beginning on line 25 has been replaced as follows:

--(i) A peptide consisting of the amino acid sequence 1-93 [of] encoded by Sequence ID No. 1 of the Sequence Table and binding calcium thereto.--.

Page 10, the paragraph beginning on line 1 has been replaced as follows:

--(ii) A peptide consisting of the amino acid sequence 1-114 [of] encoded by Sequence ID No. 2 of the Sequence Table and binding calcium thereto.--;

Page 10, the paragraph beginning on line 4 has been replaced as follows:

--(iii) A peptide having an amino acid sequence in which one or more amino acids are deleted from or added to the amino acid sequence [of] encoded by Sequence ID No. 1 or 2 of the

Sequence Table, or one or more amino acids in the amino acid sequence [of] encoded by Sequence ID No. 1 or 2 are replaced with other amino acids, binding calcium thereto, and exhibiting the activity of increasing secretion of granules of cell lines having granule secretion capability.--.

Page 11, the paragraph beginning on line 26, bridging page 12, has been replaced as follows:

--The cells having granule secretion capability separated from blood are incubated in a RPMI 1640 medium, MEM (Minimum Essential Medium) medium, or the like which contains fetal bovine serum. Suspended cells are re-suspended in the buffer solution containing potassium chloride and sodium chloride. In the case of adhered cells, supernatant of the culture liquid is discarded and cells are re-suspended in the buffer solution containing potassium chloride and sodium chloride. The suspension is incubated in the same manner as described above and processed to make the membranes permeabilized cell membranes.--.

Page 12, the paragraph beginning on line 23 has been replaced as follows:

--Treatment of cells using short electric pulses (an electroporation method) is another preferable method of forming permeabilized cell membranes. Specifically, an amount of 1×10^7 cells/ml of cell line is treated with 1-10 KV (kilovolt) electric pulses at 4-40°C for 1-30 minutes.--.

Page 16, the paragraph beginning on line 5, bridging page 17, has been replaced as follows:

--As a method of causing calgranulin to over-expression, a method of recombining a gene encoding calgranulin in a known plasmid vector or virus vector, and introducing the recombinant into the cells can be given. The [base] polynucleotide sequence shown as Sequence ID No. 1 or No. 2 in the sequence table, for example, can be used as a gene encoding calgranulin. The recombinant vector can be introduced into the cells by the calcium phosphate method, the DEAE dextran method, lipofectin method, electric pulse method, or the like. The above-described various methods may be preferably used for introducing a calgranulin gene in a cell line and causing the calgranulin to over-expression. The cells are converted to cells having the above-mentioned permeabilized cell membrane and a water-soluble calcium compound is preferably introduced in the cell line. Specifically, a calgranulin gene is introduced into cells by incubating a plasmid vector or virus vector in which the calgranulin gene has been incorporated in the amount of the 1-200 μg per 0.5×10^7 to 3×10^7 cells at 4-40°C for 5-120 minutes together with 1-100 _g of calcium phosphate, 0.1-10 mg of DEAE dextran, or 1-100 μg of lipofectin, or by treating the plasmid vector or virus vector in which the calgranulin gene has been incorporated in the amount of the 1-200 μg per 0.5×10^7 to 3×10^7 cells using a short electric pulse at 4-40°C for 1-30 minutes. The above-mentioned various methods may be used for introducing the water-soluble calcium compound.--.

Page 21, the paragraph beginning on line 6 has been replaced as follows:

--The step B) for causing the sample to contact with the cell lines having granule secretion capability may be carried out before, after, or during [the] step A) for increasing an active form of calgranulin.--;

Page 21, the paragraph beginning on line 14 has been replaced as follows:

--The same procedure as described above can be employed in the method of conducting [the] step A) to increase active form of calgranulin of cell lines having granule secretion capability. Specifically, the following methods can be given:

a) A method of converting cell membranes of cell lines having granule secretion capability, preferably neutrophils or neutrophil-like cultured cells, into permeabilized cell membranes, and simultaneously or successively adding a calgranulin and a water-soluble calcium compound.--.

Page 22, the paragraph beginning on line 8 has been replaced as follows:

--In the step B) of causing a sample which may contain a substance inhibiting or activating the granule secretion reaction to contact with the cell lines having granule secretion capability, and incubating the mixture, biological components, naturally occurring substances, compounds, and the like can be given as examples of the sample. This procedure of causing the sample to contact with the cell lines having granule secretion capability is carried out before, after, or during [the] step A) of increasing an active form of calgranulin. As the cell lines

having granule secretion capability, the said cell line having granule secretion capability itself, a cell line in which the calgranulin has been increased, a cell line in which the active form of calgranulin has been increased, and the like can be used. The former two cell lines increase an active form of calgranulin by the above-mentioned treatment for increasing the active form of calgranulin.--.

Page 36, the paragraph beginning on line 9 has been replaced as follows:

--The results are shown in Table 1. The secretion inhibiting rate of samples was determined by comparison with a control which does not contain the screening sample, assuming that the secretion from the control is 100%. Compound 1 and Compound 2 [increased] decreased the activity of calgranulin A and remarkably controlled granule secretion in a system in which the amount of elastase secretion from neutrophils has been remarkably [increased] decreased. Compound 3 remarkably increased the amount of secretion in the above system.--.

Table 1. Comparison of granule secretion activity when the order of adding calgranulin and calcium is changed.

	vehicle	calgranulin A	calgranulin B	calgranulin A & B
Method A	1	8.5	7.3	6.1
Method B	1	8.2	7.5	6.5
Method C	1	8.1	7.1	6.3

The activity of secreted elastase was determined to compare the amount of secreted elastase by 1) preparing permeabilized human neutrophil, and 2) adding calgrunulin and calcium by method A, B and C. Cakgranulin A, calgranulin B and calgranulin A & B were used as calgranulin, and permeabilized buffer alone was used as vehicle.

Preparing permeabilized human neutrophil and determining the activity of secreted elastase were achieved according to the protocol of Example 1 (See line 21 of page 30 to line 7 of page 31, and lines 15-23 of page 31, respectively).

The experiment was achieved as follows.

Neutrophil fraction prepared from human arterial blood was diluted with permeabilized buffer (30 mM HEPES, 100 mM KCl, 20 mM NaCl, 1mM EGTA, pH 7.0) to obtain neutrophil suspension ($1x10^7$ cells/ml). Digitonin was added to the suspension to a final concentration of 5-7.5 μg/ml and incubated at 37℃ for 15 minutes. After removing supernatant by centrifugation, permeabilized buffer was added to cell pellet to obtain a permeabilized human neutrophil suspension ($1x10^7$ cells/ml).

Calgranulin and calcium were added to 200 μl of permeabilized human neutrophil suspension by method A, B and C, and the suspension was further incubated at 37℃ for 5 minutes.

After supernatant was recovered, added with elastase substrate (Suc-Ala-Pro-Ala-pNA), and incubated at 37℃ for 15 minutes, absorbance at 405 nm was determined. The absorbance of vehicle of method A was assumed to be 1, and the relative secretion amount was calculated. As a result secretion of elastase is almost same between methods A, B and C. Thus it was confirmed that granule secretion was not related to the order of contact between calgranulin, calcium and cells.

Also it was re-confirmed that calgranulin becomes an active form only after contacting calcium.

Method A: Three μM of calgranulin was added to 200 μl of permeabilized human neutrophil suspension, and the suspension was incubated for 5 minutes. It was further added with 1 μM of calcium.

Method B: Three μM of calgranulin and 1 μM of calcium were added to 200 μl of permeabilized human neutrophil suspension simultaneously.

Method C: One μM of calcium were added to 200 μl of permeabilized human neutrophil suspension, and the suspension was incubated for 5 minutes. It was further added with 3 μM of calgranulin.

Table 2. Comparison of granule secretion activity when the order of adding compound is changed.

	Control	Compound 1	Compound 2	Compound 3	Compound 4
Method 1	100%	62%	53%	145%	70%
Method 2	102%	60%	51%	142%	68%
Method 3	98%	64%	52%	147%	73%

Permeabilized human neutrophil was prepared. Compounds 1-4 (a target substance in claims 19 and 27, the same with compounds 1-4 of Example 3), calgranulin A and calcium were added by method 1, 2 and 3 and the activity of secreted elastase was determined.

Preparing permeabilized human neutrophil and determining the activity of secreted elastase were achieved according to the protocol of Example 1 as described in from line 21 of page 30 to line 7 of page 31, and in lines 15-23 of page 31.

The protocol is stated in brief.

Neutrophil fraction prepared from human venous blood was diluted by permeabilized buffer (30 mM HEPES, 100 mM KCl, 20 mM NaCl, 1mM EGTA, pH 7.0) to obtain neutrophil suspension ($1x10^7$ cells/ml). Digitonin was added to the suspension to a final concentration of 5-7.5 μg/ml and incubated at 37°C for 15 minutes. After removing supernatant by centrifugation, permeabilized buffer was added to cell pellet to obtain a permeabilized human neutrophil suspension ($1x10^7$ cells/ml).

One of compounds 1-4, calgranulin and calcium were added to 200 μl of permeabilized human neutrophil suspension by method 1, 2 or 3, and the suspension was further incubated at 37°C for 5 minutes.

After supernatant was recovered, added with elastase substrate (Suc-Ala-Pro-Ala-pNA), and incubated at 37°C for 15 minutes, absorbance at 405 nm was determined. The absorbance of control of method 1 was assumed to be 100%, and the relative absorbance as the index of the secretion amount is shown in Table 2. As a result secretion of elastase is almost same between methods 1, 2 and 3. Thus it was confirmed that the effect of compound, i.e., a target substance in the claim 4 of the present application, on granule secretion was not related to the order of addition of compound, calgranulin and calcium.

Method 1:

Thirty μM of compound was added to 200 μl of permeabilized human neutrophil suspension and incubated at 37°C for 1 minute. Three μM of calgranulin and and 1 μM of calcium were added to the suspension

simultaneously.

Method 2:

Three μM of calgranulin and 1 μM of calcium were added simultaneously to 200 μl of permeabilized human neutrophil suspension and incubated at 37°C for 1 minute. Thirty μM of compound was added to the suspension.

Method 3:

Three μM of calgranulin, 1 μM of calcium and 30 μM of compound were added simultaneously to 200 μl of permeabilized human neutrophil suspension and incubated at 37°C for 1 minute.